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Using phosphate to increase feeding consumption in termite *Coptotermes formosanus*

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Abstract

Termites are ecologically significant in positive and negative ways; their role in breaking down debris greatly benefits forest environments, but this activity renders them a pest in domestic environments. This study examines the effect of nutrition on the feeding preference of termite *Coptotermes formosanus* Shiraki. Among 11 nutrition options tested, dipotassium phosphate (DKP) most significantly increased feeding consumption in the multiple feeding choice test. The mean feeding amount of the DKP-treated sample was 2.5-fold higher than that of the deionized water-treated control. This result suggests that termite colonies are deficient in phosphorus, and an additional supply of phosphate can promote feeding. The result of a no-choice feeding test using DKP shows that DKP does not promote feeding in a small number of termites. The results obtained in this study suggest that phosphorus is needed in termite colonies, making DKP especially effective. DKP will help to improve bait technology, because it is inexpensive and safe for both humans and the environment.

Keywords: Baiting, *Coptotermes formosanus*, Dipotassium phosphate, Feeding preference, Pest control

Introduction

Termites are of great ecological importance because of their ability to recycle wood and other plant materials by breaking down cellulose. However, when they began to destroy artificial wood or wooden products such as homes, buildings, and other commercial products, they become serious economic pests. Subterranean termites are one of the most important termite pests in the world with a widespread global distribution [1, 2]. Therefore, effective control methods for termites have been established and considerable efforts have been made for controlling them.

Currently, the bait system, which is a method for controlling subterranean termites, has become a common commercially developed approach [3–5]. Baiting is environmentally sound and utilizes very small amounts of

insect toxins, but these toxicants still potentially have harmful effects on other insects, such as honeybees, or mammals, including humans. Reducing the use of toxic chemicals and/or increasing the efficacy of baiting are environmentally and economically preferable.

As a means of improving baiting, several studies have examined the effects of attractants that can direct subterranean termites to the bait station. Reports on the influence of the foraging behavior of Formosan subterranean termites are as follows: wood decayed by brown rot fungi attract termites [6–8]; sucrose, yeast, or a urea solution entice termites to the drenched substance [9]; and Summon Preferred Food Source disks or its extractives improve tunneling speed and rate of infestation to stations [10, 11]. The sports drink GatoradeTM also increased the rate of tunneling [11]. Extracts from leaves of *Melaleuca leucadendra* L. attracted termites and extracts from the leaves of clove [*Syzygium aromaticum* (L.) Merr. & L.M. Perry] demonstrated trail-following activity [12].

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Increasing the feeding consumption of termites is another proven method of improving baiting efficacy. To accelerate termite feedings, the use of baits such as wood, including decayed [6, 7] or steamed [13] wood, toilet paper rolls [14], and cork [15] have proven effective. Many studies have been conducted to identify certain chemicals that increase the feeding consumption of termites. The chemicals that have been identified and patented as feeding stimulants for use in termite baiting are as follows: D-aspartic and L-glutamic acid [16], hydroquinone [17, 18], other forms of saccharides [19, 20], ergosterol [21, 22], and steroid derivatives [23]. However, in tests that compared consumption by *Coptotermes formosanus* Shiraki of filter paper disks treated with the abovementioned chemicals versus control disks over a range of concentrations, only ergosterol acted as a feeding stimulant [22]. Thus, ergosterol has been incorporated into a cellulose-based bait matrix for termite control [24].

Optimal foraging theory postulates that animals are able to choose their food according to nutritional needs to optimize fitness [25–27]. There are significant differences in the nutrient composition between termites and wood. The ash contents of wood (sapwood and main trunk) ranged from 0.2 to 2.1% [28–30], while the ash contents of termite workers reached 3.72% for *Nasutitermes* spp. [31], 9.90% for *Nasutitermes takasagoensis* (Shiraki) [32], 7.494% for *C. formosanus*, and 8.850% for *Reticulitermes speratus* Kolbe [33]. The main components of ash are phosphorus, potassium, calcium, and magnesium as well as trace amounts of metal elements (minerals) such as aluminum, iron, zinc, sodium, copper, and silicic acid. As shown by Yoshimura et al. [34], the phosphorus, sulfur, chlorine, and sodium contents in *C. formosanus* were more than tenfold higher than those of Japanese red pine (*Pinus densiflora* Sieb. & Zucc.). Hence, the author hypothesized that some (or one) of these elements are (or is) constitutively insufficient in termites or termite colonies. In addition, the effects of these elements on the feeding consumption of the subterranean termite *C. formosanus*, which is one of the most important pests, were examined.

This study aims to find novel compounds that aid effective bait use by promoting termite feeding through the addition of insufficient nutrition.

Methods

Termites

Coptotermes formosanus was used for the test in the ongoing study. Mature termites were collected on the Living-Sphere Simulation Fields (LSF) of Research Institute for Sustainable Humanosphere (RISH), Kyoto University located at Hioki City, Kagoshima Prefecture,

Japan, with pine stick traps. Termites were gently separated from the stick and immediately used in the laboratory test. Mature termites were also obtained from a laboratory colony maintained at a temperature of 28 ± 2 °C and relative humidity greater than 80% under dark in the Deterioration Organisms Laboratory (DOL) at the RISH, Kyoto University.

Materials

To test the feeding preference of termites for different nutrients, termites were confined to a sheet of nutrient-treated filter paper (qualitative filter paper model 9, AS ONE Co., Osaka, Japan) in a choice test. Initially, the filter paper was cut in 2 cm squares, with five papers layered and joined by needleless stapler (approximately 0.15 g). A paper stack was treated with one of each nutrient solution. The following reagents were used as nutrients: ammonium sulfate, calcium chloride, dipotassium phosphate (DKP), disodium phosphate, L-glutamic acid, potassium chloride, sodium chloride (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), hydroxyapatite (Kishida Chemical Co., Ltd., Osaka, Japan), 5'-inosinic acid (as inosin 5'-monophosphate disodium salt hydrate; Tokyo Chemical Industry Co. Ltd., Tokyo, Japan), and 5'-guanylic acid (Combi-Blocks Inc., San Diego, USA). The required amount or optimal concentration of nutrients for termites is difficult to predict. Nutrition solution concentrations were defined as 50 mmol/L, because general buffer concentrations ranged from 10 to 100 mmol/L in biology, and 0.3% (w/v; 50 mmol/L) NaCl were used as saline for termites, such as *C. formosanus* [35]. All chemicals used in the study, except for umami ingredients and hydroxyapatite, were tested with this concentration to determine whether they were optimum (Table 1).

A 250-μL nutrient solution was adsorbed on filter paper and dried in the oven at 105 °C for the initial weight measure. Nutrient-treated filter paper was stabbed by pin on 2.5-cm-square vinyl chloride sheets (3-mm thickness) and placed into a test container randomly.

Natural sea sand for multiple-choice feeding tests was collected from a beach in Miyazaki Prefecture, Japan. The collected natural sea sand was washed with tap water until supernatant was clear and then rinsed with deionized water. After drying by oven at 105 °C, the sand was autoclaved at 120 °C for 20 min. For no-choice feeding test, sea sand (300–600 μm; FUJIFILM Wako Pure Chemical Corporation) was used. Sea sand was autoclaved at 120 °C for 20 min before use.

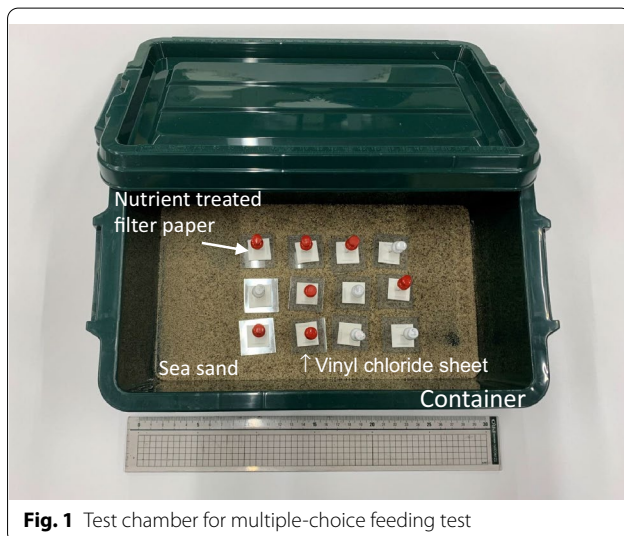
Multiple-choice feeding test

Between 3.5 and 4 g of termites (approx. 1200–1300 termites), including both workers and soldiers (soldier ratio

Table 1 Nutrition concentration used in this study

Nutrient category	Treatment	Molarity (mol/L)	Concentration (%; w/v)	Reference
Phosphorus	Dipotassium phosphate (K_2HPO_4)	0.05	0.87	
	Disodium phosphate (Na_2HPO_4)	0.05	0.71	
	5'-Guanylic acid ($C_{10}H_{14}N_5O_8P$)	0.01*	0.36	
	5'-Inosinic acid ($C_{10}H_{11}N_4Na_2O_8P$)	0.01*	0.39	
Phosphorus–calcium	Hydroxyapatite [approx. $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$]		1	
Nitrogen	Urea (CH_4N_2O)	0.05	0.30	[16]
	L-Glutamic acid ($C_5H_9NO_4$)	0.01	0.15	
Calcium	Calcium chloride ($CaCl_2$)	0.05	0.55	
Sulfur–nitrogen	Ammonium sulfate [$(NH_4)_2SO_4$]	0.05	0.66	
Salt	Potassium chloride (KCl)	0.05	0.37	[35]
	Sodium chloride (NaCl)	0.05	0.29	
–	Deionized water			

* Concentrations were adjusted to that of L-glutamic acid, since it acts as an umami ingredient

**Fig. 1** Test chamber for multiple-choice feeding test

less than 20%), were used for one trial. Seven replicates were carried out for this test. Termites were introduced into a 4-L home container ($158 \times 283 \times 95$ mm inner size, ST box #7, Astage Co. Ltd., Niigata, Japan). The container was prepared by spreading 135 g of sterilized natural sea sand containing 30 mL of deionized water in advance and placing the test samples on it (Fig. 1). After the container was covered with lid, tests were performed at 25 °C until the non-treatment control sample lost more than 20% (up to 50%) of its weight (14–25 days). During the test, deionized water was sprayed as appropriate, and the samples' position was replaced at random once a week to avoid bias in feeding by collective behavior. The filter papers were removed from the container when the test was finished and then dried in the oven at 105 °C. The weight

loss of each paper was determined. Statistical analysis was carried out using HAD software version 16 [36]. The data were analyzed by one-way analysis of variance, and the results were shown as $F(df1, df2) = F, p$, and $\eta^2 =$ partial η^2 . F means test statistic (F value) which was derived by degree of freedom ($df1$ and $df2$). p means p value in statistical testing. In addition, η^2 means correlation ratio which shows strength of association derived by partial η^2 . The method of Holm [37] was used to compare food consumption among the treated and control paper filters.

No-choice feeding test

The acrylic test tube (6 cm high by 7.4 cm diameter) was first prepared and sealed with a 5-mm-thick gypsum (New Plastone, GC Co., Tokyo, Japan) bottom covered with 10 g of sea sand containing 2 mL of deionized water, and then, test samples were placed on top. The weight of 100 workers and 10 soldier termites was measured, and the termites were introduced in an acrylic test tube. A set of treated samples and a non-treated sample were placed into a 4-L home container with a paper towel (Kimtowels, Nippon Paper Crexia Co., Ltd.) on the bottom (Fig. 2). After the container was covered with lid, tests were performed at 25 °C. During the test, dead termites were checked every 2 days, and they were removed immediately. Deionized water was sprayed as appropriate. After a 14-day period, the filter papers were removed and dried in the oven at 105 °C. The weight loss of each paper was determined. The number of surviving termites was checked, and their weight was measured. Five replicates were carried for this test. Statistical analyses were carried out by Student's t test.

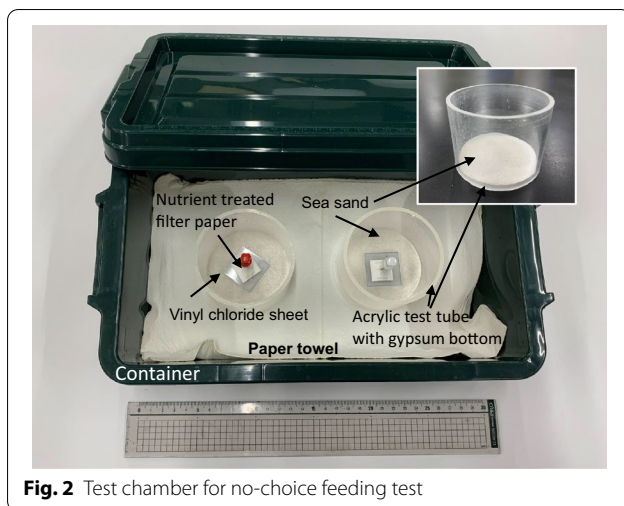


Fig. 2 Test chamber for no-choice feeding test

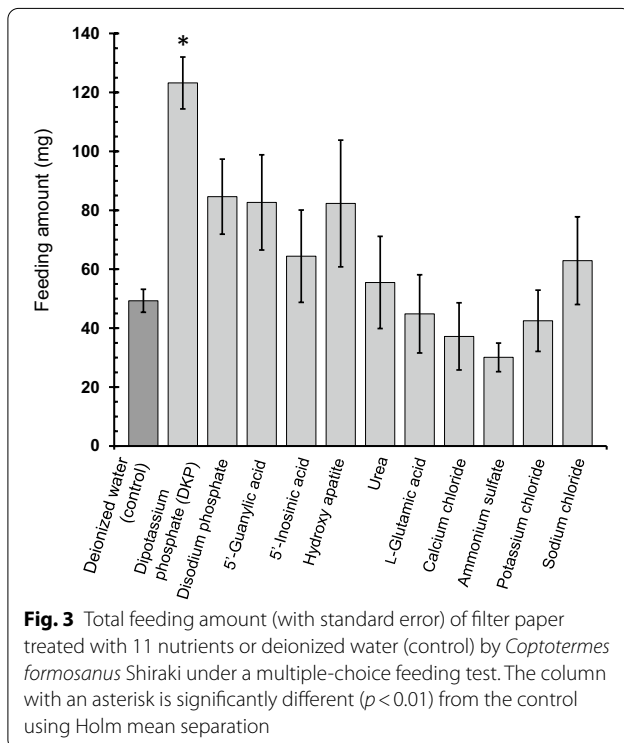


Fig. 3 Total feeding amount (with standard error) of filter paper treated with 11 nutrients or deionized water (control) by *Coptotermes formosanus* Shiraki under a multiple-choice feeding test. The column with an asterisk is significantly different ($p < 0.01$) from the control using Holm mean separation

Results

Multiple-choice feeding test

Total food consumption was significantly different between nutrient-treated samples and the control ($F [11, 66] = 4.557, p < 0.01, \eta^2 = 0.432$). Of the 11 nutrients tested, only DKP significantly increased consumption compared with the deionized water-treated control ($p < 0.01$) (Fig. 3). The mean feeding amount for the DKP-treated sample (123.2 mg) was 2.5-fold higher than that of the deionized water-treated control (49.3 mg).

No-choice feeding test

According to the results of the multiple-choice feeding test, no-choice feeding test was conducted using only DKP. In five trials, the weight losses of termites using the non-treatment control filter paper were more than 20% (up to 30%). As shown in Table 2, mean filter paper consumption in the no-choice test was not significantly different between the DKP-treated sample and the deionized water-treated control. Mean mortality and mean termite body weight fluctuation rate were also not significantly different between the treated sample and the control.

Discussions

Phosphorus (phosphate) is a nutrient essential to life for all species, including Formosan subterranean termites, as it is a component of nucleic acid. Moreover, it also acts as a primary energy source (adenosine triphosphate), a signal molecule (second messenger) of cells (cyclic adenosine monophosphate), and a coenzyme, which has critical functions in most of living things.

Results of the present study indicate that *C. formosanus* preferred filter paper containing DKP (Fig. 3). The results suggest that *C. formosanus* may feed preferentially on food sources high in phosphate and take a proactive role in supplying the colony with needed phosphate. This is also inferred by the results of the no-choice feeding test (Table 2). No significant differences were observed in feeding amount, mortality, or body weight fluctuation in the test using a restricted number of termites. It is presumed that the phosphate was saturated among a small

Table 2 Feeding amount, mortality, and termite body weight fluctuation rate of no-choice feeding test

	Treatment	Control	Difference	Paired sample <i>t</i> test <i>p</i> value
Feeding amount (mg)	39.8 ± 1.8	41.5 ± 2.5	− 1.7	0.58
Mortality (%)	8.3 ± 1.4	7.4 ± 1.5	0.9	0.66
Termite body weight fluctuation rate (%)	− 11.2 ± 3.3	− 11.9 ± 2.3	0.7	0.87

Mean values are shown with standard error. Comparison of filter paper treated with DKP and treated with deionized water (control) is shown

number (110) of termites; thus, the increase in feeding was not observed in this test.

The author's group previously reported [38] that some calcium phosphate compounds possibly accelerated decomposition of sugi [*Cryptomeria japonica* (Thunb. ex L.f.) D.Don] log wood by termites in the field. The mechanism or effective compounds were not clear at that time. From the results of the present study, the previous report seems to suggest that phosphate increases feeding of termite. Although the mean feeding amount of other phosphates including hydroxyapatite—a compound of calcium phosphate—was higher than that of the control, they were not significant. The amount of phosphate released from hydroxyapatite was considered insufficient because of its low water solubility. There is no appropriate idea about the other phosphates at this time; thus, further confirmation is needed, for example, change concentrations.

The addition of nitrogen, calcium, sulfate, and salts did not promote termite feeding in the present study. As it is unclear whether the concentration in the present study was appropriate, further study is needed to determine whether those nutrients promote termite feeding.

As mentioned in the “Introduction”, this study aimed to find novel compounds that promote termite feeding by adding insufficient nutrition, thereby helping to use baiting effectively. It is expected that our results capable for practical use in “bait” since feeding amount of DKP is 2.5-fold higher than that of deionized water-treated filter paper that equals as toilet paper roll used in bait system [14]. DKP is inexpensive, easily available from chemical companies, and commonly used as a food additive, fertilizer, and buffering agent. As a food additive, DKP is categorized by the United States Food and Drug Administration as generally recognized as safe (listed as “potassium phosphate dibasic”) [39]. Using DKPs is easier than using the previously reported certain amino acid, decayed or steamed wood and its extractives from steamed wood [6, 7, 13, 16, 40]. Furthermore, DKP can probably be used together with those amino acid, decayed wood and steamed wood or its extractives because they cannot compensate for the phosphorus deficiency. The addition of DKP will improve the efficiency of the bait system and allow a decrease in the usage of toxic chemicals.

The use of sustained release compounds, such as calcium phosphate as previously reported [38], should be considered when used under wet conditions because DKP is highly soluble in water.

Though the effect of DKP and optimal condition to use of DKP should be verified by baiting in the field, these results have a possibility that DKP is preferable for both chemical companies and the environment because chemical companies can simultaneously save baiting

drug costs and reduce the risk of environmental pollution from leaking insect toxicants.

Conclusions

The study's findings suggest that DKP is an important alternative to chemical methods for treating termite infestations. DKP effectively accelerates feeding and is inexpensive and environmentally safe. These results can improve the efficiency and efficacy of bait systems in addressing termite problems. For example, the results suggest that if DKP is impregnated, then the same level of insecticidal effect can be expected even at half the amount of the drug. Alternatively, it can effectively detect termites and reduce damage to wooden structures.

Confirming the feeding-preference activity of phosphorus to other termite species, especially subterranean termites, is necessary. Currently, another study is conducting tests using *R. speratus*, which will be reported elsewhere. Simultaneously, the future study aims to verify the effect of performing field tests by incorporating DKP into the actual bait method and realize the results of the research.

Abbreviations

DKP: Dipotassium phosphate; DOL: Deterioration Organisms Laboratory; LSF: Living-Sphere Simulation Fields; RISH: Research Institute for Sustainable Humanosphere.

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Authors' contributions

HS designed and performed the experiments, analyzed the data, and drafted the manuscript. The author agreed to be personally accountable for the contributions and to ensure that the questions are accurate and add integrity to any part of the work. The author read and approved the final manuscript.

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Availability of data and materials

All data in this study are available from the corresponding author on reasonable request.

Competing interests

The author declares no competing interests.

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